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Correlation between discharge timings of pairs of motor units reveals the presence but not the proportion of common synaptic input to motor neurons

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(e) Running title

Limitations in estimating the common input to motor neurons

(f) Index Terms:

Motor unit; inter-spike interval; short-term synchronization; common synaptic input; discharge rate.

Abstract

We investigated whether correlation measures derived from pairs of motor unit (MU) spike trains are reliable indicators of the degree of common synaptic input to motor neurons. Several 50-s isometric contractions of the biceps brachii muscle were performed at different target forces ranging from 10 to 30% of the maximal voluntary contraction (MVC) relying on force feedback. 48 pairs of MUs were examined at various force levels. Motor unit synchrony was assessed by cross-correlation analysis using three indices: the output correlation as the peak of the cross-histogram (ρ), the number of synchronous spikes per second (CIS), and per trigger (E). Individual analysis of MU pairs revealed that ρ, CIS, and E were most often positively associated to discharge rate (87, 85, and 76% of the MU pairs, respectively) and negatively to inter-spike interval variability (69, 65, and 62% of the MU pairs, respectively). Moreover, the behaviour of synchronization indices with discharge rate (and inter-spike interval variability) varied greatly among the MU pairs. These results were consistent with theoretical predictions, which showed that the output correlation between pairs of spike trains depends on the statistics of the input current and motor neuron intrinsic properties that differ for different motor neuron pairs. In conclusion, the synchronization between MU firing trains is necessarily caused by the (functional) common input to motor neurons, but it is not possible to infer the degree of shared common input to a pair of motor neurons based on correlation measures of their output spike trains.

Index terms: motor unit; inter-spike interval; short-term synchronization; common synaptic input; discharge rate.

Running title: Limitations in estimating the common input to motor neurons

New & Noteworthy

The strength of correlation between output spike trains is only poorly associated with the degree of common input to the population of motor neurons

The synchronization between motor unit firing trains is necessarily caused by the (functional) common input to motor neurons, but it is not possible to infer the degree of shared common input to a pair of motor neurons based on correlation measures of their output spike trains.

1. INTRODUCTION

During sustained contractions, motor neurons receive both common and independent synaptic inputs from presynaptic neurons and supraspinal centers (Ishizuka et al. 1979; Lawrence et al. 1985; Lemon 2008). The joint occurrence of excitatory post-synaptic potentials generated by branching common inputs alter the membrane potentials of different motor neurons in a correlated way, momentarily raising the joint probability of firing concurrently (Sears and Stagg, 1976; Kirkwood and Sears 1978; Farmer et al. 1993). As a result, there is a tendency for pairs of motor units (MUs) to discharge within a few milliseconds of one another, which has been termed short-term synchronization. The cortico-spinal pathway is considered as the main generator of synchronizing inputs, but there are other contributing sources, such as the spinal inhibitory interneurons (Kirwood et al. 1982). Therefore, measurements of the amount of shared input would provide valuable information about the connectivity between the motor neuron pool and spinal or cortical networks. However, direct quantification of the strength of common input that the motor neurons receive has not yet proved feasible. Rather, shared input is normally estimated indirectly by analysing the output behaviour of motor neurons, i.e. the discharge timings of the corresponding MUs.

Traditional methods for estimating the amount of common input to the motor neuron pool are commonly based on the assessment of correlation between pairs of output spike trains discharged by MUs (Ellaway 1978; Nordstrom et al. 1992). The main assumption of these methods is that the correlation between output spike trains is an invariant index of the degree of common input that the motor neurons receive (see Kirkwood et al. 1979 for a review). This assumption, however, is invalid since the output correlation does not only depend on the input correlation, but it is also a function of the mean and variance of the current injected into the neuron (de la Rocha et al. 2007). Since the statistics of this input current cannot be reliably estimated in vivo, the relation between the output (measured) correlation and input (actual) correlation is uncertain.

An important consequence of the dependence of the output correlation strength on the statistics of the synaptic input current is that the correlation measures between output spike trains depend on the discharge rate, even for a constant degree of correlation of synaptic input (Negro and Farina 2012). Although the importance of the bias introduced by the discharge rate has been acknowledged by several authors (Schmied et al 1994; Semmler et al., 2004), its specific effect has not yet fully recognized as an intrinsic feature (and limitation) of the correlation measures and so it remains poorly understood. For example, it is often assumed that the effect of discharge rate can be removed by applying normalizing methods to the correlation indices (Türker and Powers 2001; Schmied and Descarreaux 2010). However, normalization cannot suppress completely the discharge rate effect. The reason is that the oscillations of the synaptic current are not correctly reconstructed due to the low discharge rates of alpha motor neurons (for details see Negro and Farina, 2012). As a result, a deficient “transmission” of the synaptic input current by individual motor neurons occurs. The implication is that it is impossible to judge whether the changes in the output correlation measures are due primarily to actual variations in the input correlation strength or to variations in the statistics and amount of synaptic current.

An additional confounding factor that complicates the estimate of input correlation is the variability of the inter-spike interval (ISI) (see the theoretical analysis in the Methods section). This factor may change depending on the experimental conditions in which synchronization is investigated. In experiments with visual and/or auditory feedback of at least one of the activated MUs (Datta and Stephens 1990; Nordstrom et al. 1992; Christou et al. 2007), the variability of the ISI is indeed usually greater than in behavioural tasks with force feedback, in which the subjects are not aware of the MU activity underlying force production (Fling et al. 2009: Schmied and Descarreaux 2010; Negro and Farina 2012). Moreover, the correlation between MU discharges may be altered by feedback on individual MUs and therefore may not represent the natural conditions (Mori, 1975; Kamen and Roy, 2000). Finally, ISI variability also depends on the synaptic input current (de la Rocha et al. 2007) and, as a result, its effect on the output correlation cannot be easily removed.

The complex non-linear nature of the relation between the output (measured) correlation and input (actual) correlation has led to a debate on the existence of common input. For example, recently, Kline and De Luca (2016) argued that synchronicity of MU activation may not be caused by common inputs to motor neurons; rather, they judged synchronization as an epiphenomenon, due to unknown neural interactions. This conclusion was also based on a previous observation by the same authors that, when analysed with rigorous statistical methods, only a relatively small portion of MU pairs actually showed a significant correlation of their spike trains (De Luca and Kline, 2014). With the present study, however, we provide evidence that the output correlation between pairs of spike trains is not strongly associated to, despite being caused by, common input. Therefore, lack of correlation in output spike trains for a pair of MUs does not necessarily imply lack of common input between the corresponding motor neurons. It is underlined that we consider here functional common input, i.e. the correlation between inputs to different neurons, which is not necessarily structural, i.e. the correlation may be generated by different sources rather than due to anatomical common projections. We maintain this assumption since any measures of correlation between MU spike trains cannot identify the way in which common input is generated.

We report the theoretical fundamentals of the relation between the correlation in synaptic input and the correlation between pairs of MU discharge timings. These theoretical derivations are then verified experimentally by analysing the dependence of the output correlation on discharge rate and ISI variability. The experimentally identified relations as well as the large variability in correlation measures observed among different MU pairs confirmed the theoretical analysis. Moreover, individual and group analysis of MU pairs indicated that the output correlation between pairs of spike trains is a reflection of common input to motor neurons, although common input and output correlation are not linearly related. The results showed that correlation measures estimated from pairs of MU spike trains are not reliable indicators of the actual level of correlation in synaptic input.

2 MATERIAL AND METHODS

2.1 Theory

A mathematical expression has been previously proposed to relate the strength of common synaptic input and the correlation between output spike trains in cortical neurons (de la Rocha et al. 2007). According to this derivation, the input current delivered to a population of motor neurons can be described as:

$$I_i = \mu_i + \sigma_i\left[\sqrt{1-c}\cdot\xi_i(t) + c\cdot\xi_c(t)\right] \quad (1)$$

where c, μ_i, and σ_i are the input correlation, the mean, and the standard deviation of the synaptic input current, respectively, for each motor neuron *i*. $\xi_i(t)$, and $\xi_c(t)$ represents realizations of independent and common Gaussian noises, respectively. According to Eq. (1), the input correlation can be interpreted as the proportion of variance of a common synaptic input that is shared by different motor neurons. As described by de la Rocha et al. (2007), the output correlation between spike trains of two neurons (ρ) is associated to the correlation in their synaptic inputs (c) by the following expression:

$$\rho = \frac{\sigma\left(\frac{d(DR)}{d(\mu)}\right)^2}{(CoV\_ISI)^2 DR}\cdot c = S(DR)\cdot c \quad (2)$$

where μ and σ are, respectively, the mean and the standard deviation of the input current, DR and CoV_ISI are, respectively, the average discharge rate of the neurons and the coefficient of variation for the interspike interval, and $\frac{d(DR)}{d(\mu)}$ indicates the first derivative of discharge rate with respect to the mean input current.

The synchronization indices proposed in the literature, extracted from pairs of MU discharge timings, provide estimates of the output correlation (ρ) whereas the input correlation c is not accessible. Eq. (2) indicates the association between the variable that can be directly estimated (ρ) and the actual input correlation (c), which is the unknown physiological strength of common input (Fig. 1). This equation shows that the estimate ρ depends on several parameters other than c and, as a result, ρ is not an invariant index of the input correlation c (i.e., an increases of c is not necessarily followed by an increase in ρ). In particular, the estimate ρ depends on the statistics of the input current (μ and σ) as well as on the discharge rate and ISI variability (DR and CoV_ISI). Because the statistics of the input current cannot be reliably measured in vivo, an intrinsic uncertainty exists in the relation between ρ (measured correlation) and c (actual input correlation). Nonetheless, Eq. (2) also indicates

that ρ is different from zero only if c is different from zero. Therefore, the observation of correlation between output spike trains necessarily implies a non-zero proportion of common input.

In Eq. (2) the output correlation is expressed as the product of a scaling factor $S(DR)$ and the input correlation. This scaling factor highlights that the dependence of the output correlation on the discharge rate is a consequence of the spiking nature of the neurons, specifically of the inability of motor neurons to "sample" (i.e., to follow) correctly the oscillations of the synaptic current. This results in an intrinsic dependency of the correlation measures on the discharge rates of motor neurons (Negro and Farina 2011; Negro and Farina 2012). Two common indices of output correlation, the Common Input Strength (CIS) and the Synchronous Impulse Probability (E), are derived from ρ by using normalization methods and can be expressed as follows:

$$\rho\_CIS = S'(DR)\cdot c \tag{3}$$

$$\rho\_E = S''(DR)\cdot c \tag{4}$$

where $S'(DR)$ and $S''(DR)$ are the scaling factors that are obtained by the normalization applied to the two indices [see Nordstrom et al. (1992) and Data and Stephens (1990), respectively].

— FIGURE 1 about here —

In the experimental part of this study we aimed at validating experimentally the theoretical derivations of Eqs. (2-4) by studying the dependency of the output correlation (estimated as ρ, CIS, and E) on measurable MU properties, specifically discharge rate and ISI variability. It was hypothesized that (1) the three correlation indices studied here will show significant associations with discharge rate and ISI variability and that (2) these associations will vary substantially from one MU pair to another. If these hypotheses were confirmed, it would imply that inferring the strength of common input from MU pairs is not appropriate.

2.2 Experiments

Subjects

Ten healthy subjects (nine men and one woman) participated in the study (mean ± SD, age: 29.2 ± 3.4 years; height: 173 ± 6 cm; weight: 71 ± 7 kg; mean thickness of the subcutaneous layer over the biceps brachii, as estimated by ultrasonography: 5 ± 1 mm). None of the subjects reported symptoms of neuromuscular or ligament disorders. The study was conducted in accordance with the Declaration of Helsinki and approved by the local Ethics Committee of the Region North Jylland, Denmark; prior written informed consent was obtained from all participants.

Procedures

Participants were seated comfortably in an adjustable chair with their right arm constrained in a device designed to measure isometric elbow flexion force. During the contractions, the elbow rested on a support and was flexed at 90º, and flexion forces were exerted in the sagittal plane. The forearm was secured in place by two wide Velcro straps so that it remained in a supinated position throughout the experiment. Visual feedback on the elbow flexion force was provided on an oscilloscope.

The maximal voluntary contraction (MVC) torque in flexion was recorded three times with 3-min rests in between MVCs. The highest MVC measure was the reference for the definition of submaximal torque contraction levels. After the MVC measure, intramuscular and surface electrodes for electromyogram (EMG) signal detection were mounted on the right biceps brachii, as described below. The elbow flexion force was displayed on-line on an oscilloscope facing the subject. The subjects were asked to sustain the force level on a target that was visually presented as a horizontal line at the mid level of the oscilloscope screen. Isometric contractions of 60-s duration were performed at target forces of 10, 15, 20, 25, and 30% MVC. The force target was adjusted in random order between the lowest and the highest levels of force. A 2-min resting period was allowed between contractions to minimize the influence of fatigue. Contraction levels greater than 30% were avoided as they normally corresponded to high levels of MU activation, thereby compromising the reliability of the MU discrimination process from the intramuscular EMG signals. The total elbow flexion force was presented to the subjects without any feedback of MU discharge rate.

EMG recordings

Surface and intramuscular EMG signals were recorded concurrently from the long head of the right biceps brachii muscle. The intramuscular EMG signal was used to detect the discharge times of individual MUs and this information was used to obtain the corresponding surface MUPs by spike-triggered averaging on the raw surface EMG signal.

Surface EMG signals were recorded by a two-dimensional (2D) adhesive grid (SPES Medica, Salerno, Italy) of 13 × 5 electrodes (each of 1-mm diameter and with an inter-electrode distance of 8 mm). To determine an optimal placement of the 2D grid, the position of the innervation zone and tendon regions were detected with

the assistance of a linear non-adhesive grid. The 2D grid was then located between the proximal and distal tendons of the biceps brachii, with the electrode columns (comprising 13 electrodes) oriented along the muscle fibers and with the seventh row approximately coinciding with the innervation zone (for details, see Rodriguez-Falces et al. 2013). In order to leave space for the insertion of the intramuscular needles, an opening was made in the grid at the level of the sixth row. Surface EMG signals were detected in monopolar configuration and amplified (EMG amplifier, EMG-USB, OT Bioelettronica, Torino; bandwidth 10–500 Hz; gain 1000), sampled at 2048 Hz, and stored after 12-bit analog-to-digital conversion. Recording of surface EMG signals allowed to extract the representation of each MUP on the 2D grid, which provided valuable information to verify that the same MU was analysed across different force levels (see below).

Single motor unit action potentials were recorded from the right biceps brachii muscle with Teflon-coated stainless steel wires (diameter 0.1 mm; A-M Systems, Carlsborg, WA) inserted into the muscle with 25-gauge hypodermic needles. To increase the sampling size of the MU population, two needles of different lengths (15 mm and 25 mm) were inserted approximately 10 mm apart in the transverse direction in the proximal portion of the muscle. To further increase the number of detected MUs, three wire electrodes made of Teflon-coated stainless steel (A-M Systems, Carlsborg, WA) were mounted inside each needle. Approximately 1-2 mm of each wire was un-insulated at the tip to detect intramuscular EMG signals. Needles were removed and the wire bundle remained lodged inside the muscle. For each needle, the two bipolar intramuscular EMG signals were amplified (Counterpoint EMG, Dantec Medical, Skovlunde, Denmark, gain 500), band-pass filtered (500 Hz to 5 kHz), and sampled at 10 kHz. The position of the wires was slightly adjusted before starting the recordings and when the signal quality was poor, which occurred rarely, new wires were inserted.

Single motor unit analysis

Intramuscular EMG signals were decomposed into MU discharge trains by the use of an interactive decomposition algorithm, EMGLAB (McGill et al. 2005), which includes a user interface for manually editing and verifying the accuracy of the spike trains. Once automatic discrimination was completed, each MU spike train was manually edited by an experienced operator and inspected for checking potential discrimination errors. Specifically, all very long (>250 ms) or short (<20 ms) ISIs were inspected and excluded if decomposition errors were identified. From the decomposition analysis, spike trains of individual MUs were obtained with a sampling rate of 1000 Hz.

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templates of the identified MUAPs have been subtracted from the interferential signal) of the intramuscular EMG signal. The software used for the decomposition provided the possibility to check visually the accuracy of the identification by comparing the original EMG signal and the residual after the subtraction of the identified MUAPs. This procedure was performed until the residual was comparable to the baseline noise level of the signal. When the power of the residual signal was not at noise level, which occurred rarely (1.9 ± 0.4 % of the identified discharges), it was assumed that decomposition errors were present and these instances were manually inspected. This manual check for decomposition errors was thus irrespective of the value of the estimated ISI. In most cases, the inspected decomposition errors corresponded to discharges with ISI >250 ms or <20 ms.

To ensure that the same MU was being tested throughout the different levels of force, the reproducibility of size and shape of the associated intramuscular and surface MUPs was examined. For the intramuscular MUPs evaluation, the potential averaged across the recording period at a given force level was calculated and compared with the average potentials obtained at other force levels using cross-correlation analysis [see Fig. 2(b) and (c) for an example]. Only MUs that showed intramuscular MUPs across different force levels with a peak of the cross-correlation function greater than 0.85 were accepted for further analysis. As for the surface MUP evaluation, the representation of the MU over the skin surface was extracted by the spike-triggered averaging technique using events of the MU spike trains as triggers throughout each of the recording periods. The similarity between surface MUPs extracted at different force levels was also verified by cross-correlation analysis with a minimum threshold of 0.85.

Motor unit spike trains were characterized by computing the average discharge rate in successive, non-overlapping, 5-s intervals. The mean value of discharge rate for a given MU spike train was then calculated as the average across each of the 5-s steps. The discharge rate for a given MU pair was calculated as the geometric mean of the discharge rates of the associated MU trains. The variability of the interspike interval for a given MU spike train was determined by calculating the coefficient of variation of the interspike intervals measured over the 5-s steps. Then, the CoV_ISI for a given MU pair was computed as the geometric mean of the interspike interval variability of the corresponding MU trains. ISIs longer than 250 ms were considered as reflecting pauses in MU tonic activity and so they were excluded from the calculation of discharge rate statistics.

Synchronization indices

The magnitude of synchronization was quantified using the cross-correlation function of the raw spike trains (equivalent to a bin width of 1 ms duration, given a sampling frequency of 1000 Hz) (Nordstrom et al. 1992). For each MU pair, the cross-correlation histogram was built by comparing each discharge time of the reference MU with all discharge times of the other unit within ± 100 ms [Fig. 2(d)]. As in previous studies (Semmler et al. 2004), the MU discharging at the lower rate was considered the reference MU. The cumulative sum (CUSUM) technique (Ellaway 1978), which consists of detecting the rising inflection occurring in the central region of the

CUSUM function, was used to determine the location of the peak of the histograms [Fig. 2(d)]. The baseline of the histogram, computed as the average over the first 50 bins, represented the number of counts expected by chance, whereas the magnitude of the peak, calculated as the number of counts in the peak above the baseline, indicated the amount of synchronous firings. Histograms with a peak count less than 4 were not analysed (Semmler et al. 1997).

The strength of short-term synchronization in MU spike trains, as classically defined, was evaluated on the basis of the cross-correlogram peak magnitude by means of three indices, namely, the output correlation (ρ), the Common Input Strength (CIS, Nordstrom et al. 1992), and the Synchronous Impulse Probability (E, Datta and Stephens 1990). The CIS index was computed as the number of synchronous discharges of the MU pair in excess of chance, divided by the duration of the analysed interval, whereas the E index was defined as the number of counts in the peak above chance divided by the number of reference spikes used to compute the cross-correlation function.

Statistics

Statistical analysis was performed to examine the dependence of synchronization measures on discharge rate, on the difference in discharge rate between motor neurons in a pair, and on ISI variability (CoV_ISI). Statistical tests were performed separately for each MU pair as well as for the pooled data from all MU pairs. For the individual MU pairs examination, linear regression analysis was performed and the Pearson correlation coefficient (R), to assess the degree of correlation between the above variables, was computed.

For the aggregate data from all MU pairs, linear regression analysis was also performed to assess the degree of correlation between the MU synchronization indices and discharge rate, the difference in discharge rate between motor neurons, and CoV_ISI. In this pooled analysis, the data of the average discharge rate and CoV_ISI associated to each of the MU pairs obtained at the different force levels, together with the corresponding synchronization indices (ρ, CIS, and E), were included. To elucidate possible trends observed with linear regression analysis for each MU pair, synchronization values corresponding to the lowest and highest discharge rate, contraction level, and CoV_ISI were computed. Differences in the MU synchronization indices assessed at maximum and minimum discharge rate, contraction level, and CoV_ISI were compared by Student's paired t-tests (two-tailed) across the whole the population of MU pairs identified. In addition, multiple regression analysis was performed, where the dependent variables were the synchronization indices (ρ, CIS, and E) and the independent variables were discharge rate, the difference in discharge rate between motor neurons, and CoV_ISI. In addition, the degree of association among the studied synchronization indices (ρ, CIS, and E) was also assessed. Significance was accepted for P values smaller than 0.05.

3. RESULTS

A total of 61 pairs of MUs were identified for at least 3 force levels (ranging from 10% to 30% MVC). Thirteen MU pairs were, however, excluded because of excessive variability in their associated intramuscular MUPs across the force levels tested. Among the 48 remaining MU pairs, 24 were tracked across 3 contraction levels, 18 were followed over 4 levels, and 6 MU pairs could be identified across 5 force levels. A total of 174 recording sequences with durations of 38 ± 6 s were analysed for extracting the corresponding MU discharge timings. The average number of triggers per MU train was 480 ± 17. Among the 174 cross-correlograms analysed, only 12 did not exhibit a central peak (7.0%). These "flat" cross-correlograms were found in equal proportion throughout all force levels. The average width of the peaks was 8.97 (3.3) ms.

Figure 2 shows the surface EMG signals [Fig. 2(a)] and discharge rates of a pair of MUs, MU1 and MU2 [Figs. 2(b) and (c), respectively], during 60-s isometric contractions at five contraction forces from 10% to 30% MVC. The intramuscular action potential associated to each firing train is shown above the discharge rate plot in order to provide evidence that the same MU was presumably detected across the five contraction forces. Matching between the shapes of the multi-channel surface MUPs corresponding to different force levels was additionally performed to provide more certainty in the identification of the MU. Fig. 2(e) shows the average discharge rates of MU1 and MU2 as a function of force level. In this example, there was an increase in discharge rate with increasing contraction force, although this increase was not monotonic (discharge rate was greater at 15% than at 20% MVC).

For each contraction level, the cross-correlation histogram and the associated CUSUM diagram between the firing trains of MU1 and MU2 were constructed to determine the degree of synchronization between the two MUs [Fig. 2(d)]. The histograms had an oscillating baseline which exhibited a peak centred at time lag = 0 ms. The width of this peak was determined by the steep rising phase of the CUSUM diagram. The magnitude of the peak gave an indication of how many times MU1 discharged in synchrony (i.e., above the number expected by chance) with MU2. The estimated synchronization between MU1 and MU2 increased with discharge rate, although this increase was not monotonic [Fig. 2(f)]. In addition, in this example, the synchronization between MU1 and MU2 tended to decrease as the coefficient of variation of the interspike-intervals (CoV_ISI) of their associated discharge trains increased [Fig. 2(g)].

— FIGURE 2 about here —

Over the population of tracked MUs, the increase in contraction force corresponded to an increase in excitatory drive to the motor neurons. Fig. 3(a) shows the motor neuron discharge rate as a function of force for the 48 MUs tracked, and Fig. 3(c) reports the changes in EMG RMS with force for the 10 subjects. Increasing the force level was associated with rises in the EMG activity and increases in the discharge rates of most MUs,

thereby reflecting greater excitatory drive. Note also that CoV_ISI tended to decrease with increasing contraction force [Fig. 3(b)].

— FIGURE 3 about here —

Analysis of individual MU pair data

Fig. 4 shows the variation of output correlation (ρ) as a function of discharge rate (first row) and CoV_ISI (second row) for MU pairs tracked across 3, 4, and 5 force levels. Although the changes of ρ with discharge rate showed a large variability among MU pairs, a positive relation between ρ and discharge rate was found for 87% of the pairs examined. Conversely, the output correlation tended to decrease with CoV_ISI (69% of the pairs).

— FIGURE 4 about here —

The dependence of synchronization indices on the discharge rate was examined separately for each of the MU pairs. Fig. 5 shows the CIS values as a function of discharge rate for each of the 48 MUs tracked (first row), together with their associated linear regression lines (second row). Although the behaviour of CIS with discharge rate varied greatly among MU pairs, in general, a positive relation existed between these two variables (85% of the MU pairs). Note that, in most instances, the changes in CIS were not monotonic, i.e., CIS did not co-vary consistently with discharge rate. In addition, there was a large variability in CIS values between MU pairs. The variability in the CIS-discharge rate relation is evident in the different slopes (m) of the regression lines shown in Fig. 5 (second row). The behaviour of the other synchronization index analysed (i.e., E) with discharge rate was very similar to that of CIS. Specifically, a positive correlation between E and discharge rate was found for 76% of the MU pairs.

— FIGURE 5 about here —

The effect of CoV_ISI on synchronization indices was also investigated individually for each MU pair, as shown in Fig. 6. There was a high variability in the behaviour of CIS with CoV_ISI among pairs of MUs. There was a greater number of MU pairs for which CIS and CoV_ISI were negatively associated (65% of the pairs). The negative relation between CIS and CoV_ISI was predominant among MU pairs that were tracked across 4 and 5 contraction levels. The behaviour of the synchronous impulse probability (E) with CoV_ISI was very similar to that of CIS, with a negative correlation between E and discharge rate found in 62% of the MU pairs.

— FIGURE 6 about here —

The Pearson correlation coefficient (R) associated to the relation between CIS and discharge rate was calculated for each MU pair and then grouped into the histogram shown in Fig. 7(a). It can be seen that the distribution of Pearson coefficients was skewed to the left, with approximately 85% of the coefficients being positive, indicating that CIS usually increased with discharge rate. Likewise, the Pearson coefficient associated to the relation between CIS and the difference in discharge rate between motor neurons was calculated for each MU pair and then grouped into the histogram shown in Fig. 7(c). This histogram reveals that about 73% of the coefficients were positive, indicating that CIS was positively related to the difference in the discharge rate of the neurons. The correlation coefficients associated to the relation between CIS and CoV_ISI were also calculated and presented in the histogram of Fig. 7(b). For this relation, the correlation coefficient had a distribution skewed to the right, reflecting a slightly greater number of negative coefficients (65%). Although not presented here, the behaviour of the other synchronization indices (ρ and E) with the motor neuron properties (discharge rate, difference in discharge rate, and CoV_ISI) was similar to that observed for CIS.

— FIGURE 7 about here —

These results evidenced that the three measures of output correlation (ρ, CIS and E) had a significant positive dependence on discharge rate and on the difference in discharge rate between motor neurons, while a significant negative dependence on ISI variability. The results also show that these relations had a high variability among MU pairs. The large variability found is due to the fact that the output correlation also depends on the statistics of the input current, which varies among the pairs investigated.

Analysis of pooled MU data

Changes in synchronization indices with discharge rate were also examined for the whole population of MUs, across all subjects and forces. Regression analysis performed upon the pooled data confirmed the positive correlation between the output correlation (ρ) and discharge rate [Fig. 8(a), $R = 0.418$, $P < 0.001$]. In agreement, both synchronization indices (CIS and E) were positively correlated with discharge rate ($P < 0.001$ and $P = 0.044$, respectively).

— FIGURE 8 about here —

The pooled data analysis revealed a significant negative correlation between the synchronization indices and the coefficient of variation of ISIs [for ρ, R = -0.23, Fig. 9(a); for CIS, R = -0.27, Fig. 9(b); for SIP, R = -0.22, Fig. 9(b)]. However, no significant association was found between the synchronization indices and the difference in discharge rate between pairs of motor neurons [for ρ, R = 0.02 and $P = 0.58$; for CIS, R = -0.07 and $P = 0.32$; for SIP, R = 0.03 and $P = 0.64$].

— FIGURE 9 about here —

To further investigate the effect of discharge rate, ISI variability, and contraction strength on synchronous activity, we selected, for each MU pair, the ρ, CIS, and E values obtained at the lowest and highest discharge rate (Table 1, left column), lowest and highest force level (Table 1, central column), and lowest and highest CoV_ISI (Table 1, right column). As expected, the CIS and E indices were significantly greater when the MUs discharged at higher discharge rates, with stronger EMG activity, and at greater forces. In addition, slower discharge rates were associated with lower EMG and force values as well as greater ISI variability (CoV_ISI).

— TABLE 1 about here —

Multiple regression analysis revealed that almost 20% of the variance in CIS was explained by discharge rate and CoV_ISI (Table 2), but that the difference in discharge rate between pairs of motor neurons did not significantly influence CIS. Likewise, discharge rate and CoV_ISI together explained part of the variance of ρ and SIP (15% and 10%, respectively), while the difference in discharge rate of motor neurons did not influenced the two indexes (Table 2).

— TABLE 2 about here —

It is often believed that different normalizations lead to different behaviour of synchronization indices, e.g. as a function of discharge rate. However, when all data were pooled together, the three indices investigated showed very high positive correlation, ranging from 0.73 between E and ρ to 0.86 between E and CIS (Fig. 10). This result further indicates that normalization does not have a profound effect on the quantification of synchronization from the cross-histogram of MU pairs. The simple peak of the histogram (ρ) provides similar information as normalized values.

— FIGURE 10 about here —

The values of synchronization indexes obtained when using only the results of the automatic decomposition without manual exclusion of the erroneous discharges (decomposition errors) were not significantly different from those obtained after the decomposition errors were excluded ($P>0.05$). Moreover, the values of correlation between the MU synchronization indices and discharge rate and CoV_ISI were similar before and after excluding the discharges with decomposition errors ($P>0.05$).

4. DISCUSSION

The main findings of this study are: (1) the measures of output correlation investigated (ρ, CIS, and E) were positively related to discharge rate and negatively related to ISI variability; (2) individual analysis of MU pairs revealed that the behaviour of correlation measures with discharge rate (and ISI variability) varied greatly among MU pairs and that, for each MU pair, an increase in discharge rate was not always accompanied by an increase in the correlation measures; (3) despite the different normalization procedures for the indices analysed, their values were highly positively correlated across the conditions analysed.

4.1 Dependence of motor unit synchronization on discharge rate and inter-spike interval variability

The analysis of individual MU pairs revealed that ρ, CIS, and E increased with discharge rate in 87, 85, and 76% of the cases, respectively. This positive dependence was confirmed by the regression analysis across the whole MU population, which showed that discharge rate was positively related with all synchronization indices tested. These findings are in agreement with the theoretical formulations presented in section 2.1, which predicted that measures of output correlation should be proportional to the rate of increase of discharge rate with input current [the term $(dDR/d\mu)^2$ in Eq. (2)]. Therefore, the present results support our first hypothesis that discharge rate exerts a positive influence on output correlation measures and are in agreement with the findings by Schmied and Descarreaux (2010).

Previous works studying the behaviour of MU correlation measures as a function of discharge rate have yielded conflicting results. These discrepancies might be explained by differences in the experimental designs used. For example, in those studies where the subjects relied on audio-visual feedback of the MU firings to maintain a steady discharge rate, CIS was found to be independent on MU discharge rate (Nordstrom et al. 1992; Schmied et al. 1993, 1994; Christou et al. 2007). This feedback approach, however, does not measure the natural behavior of motor units and may alter the natural firing properties (Mori, 1975; Kamen and Roy, 2000). In fact, under more natural physiological conditions (i.e., force output controlled without any feedback on the MU firing pattern), CIS was observed to increase with discharge rate (Türker and Powers 2002; Schmied and Descarreaux 2010; Negro and Farina 2012), in agreement with our results. Another factor that might have obscured the effect of discharge rate on correlation measures is the fact that, traditionally, synchronization analysis was based on data from MU pairs pooled from the same or different subjects. Since the level of synchronization vary greatly among different MU pairs (Bremner et al. 1991; De Luca et al. 1993), aggregating data from multiple MU pairs extracted from different subjects is likely to prevent the detection of a clear dependence of the correlation measures on discharge rate. For example, Schmied and Descarreaux (2010) did not observed any significant relation between E and discharge rate in pooled data whereas they identified this relation when the MUs were analysed individually.

Since the number of synchronous discharges depends on the total number of spikes in the spike trains of two MUs, a number of methods have been proposed to normalize the number of synchronous counts. The common

purpose of these methods of normalization is to derive an index of synchronization that provides a quantitative measure of the shared input that is independent of the background discharge rate of the two MUs, that is, a more direct estimate of c [Eq. (1)]. As an example, Nordstrom et al. (1992) supported the view that the CIS index is mathematically independent of motor neuron discharge rate, which received further support from other experimental works (Schmied et al. 1993, 1994; Christou et al., 2007). Conversely, other authors claimed that the CIS index is actually, by definition, biased by discharge rate (Türker and Powers 2002; Fling et al. 2009; Schmied and Descarreaux 2010). These same authors argued that, since the E index represents the amount of synchronous firings per trigger, it should be independent of how fast the motor neuron "triggers". However, by examining the changes in synchronization separately for each MU pair, we have demonstrated that both CIS and E are positively correlated with discharge rate. It is important to emphasize that any measure of correlation based on cross-correlograms (i.e., ρ, CIS, and E) can be seen as an estimate of the actual input correlation (c), on the basis of Eq. (2). However, as predicted by this equation, the output correlation (ρ) is related to the input correlation by the scaling factor S(DR), which depends non-linearly on the discharge rate. Moreover, in this scaling factor, discharge rate also varies with the input current. Consequently, the factor S(DR) cannot be eliminated from the relation between ρ and c. As a result, none of the normalization methods are effective in compensating for the factor S(DR) in Eq. (2). In addition, the factor S(DR) depends on the motor neuron investigated (because the statistics of the input current vary from neuron to neuron), which implies that normalized indices may work for some MU pairs but not for others. Further evidence that the different normalization methods did not influence the dependence of the estimated synchronization levels on the factors of influence is that a relatively high positive correlation between the three synchronization indices was found when all data were pooled together (Fig. 10).

Individual analysis of MU pairs also indicated a tendency for a negative correlation between estimates of synchronization and ISI variability. Further support for this negative relation comes from the regression analysis across the whole MU population. These results are in agreement with the theoretical predictions described in section 2.1, which show that the output correlation is inversely proportional to CoV_ISI. This observation is opposite to some previous studies that reported a positive association between CoV_ISI and both CIS and E (Nordstrom et al. 1992; Semmler et al. 2000). This discrepancy, however, may also be linked to differences between experimental protocols where subjects have feedback on MU activity (Datta and Stephens 1990; Nordstrom et al. 1992; Christou et al. 2007) and those where subjects receive feedback on force (Fling et al. 2009: Schmied and Descarreaux 2010; Negro and Farina 2012). Moreover, there is an intrinsic variability of the results depending on the MU sample, as discussed in the following.

4.2 Variability across MU pairs

By tracking individual MU pairs at different force levels we demonstrated that, although synchronization indices and discharge rate were positively correlated, an increase in discharge rate was not always paralleled by an increase in ρ, CIS, and E. This non-monotonic increase of correlation measures with discharge rate (and thus

force) can also be appreciated in the study by Schmied and Descarreaux (2010). Importantly, we found that the variance in the relationship between output correlation and discharge rate was not explained by the difference in the discharge frequency of the neurons. Similarly, an increase in ISI variability was not systematically accompanied by a decrease in ρ, CIS, and E. Therefore, ρ, CIS, and E must depend on factors other than the discharge rate and CoV_ISI, such as the variance of the synaptic input current (σ), as predicted by the theoretical formulations. Indeed, it is most likely that in our experimental protocol the increases in voluntary drive induced changes in the statistics of the synaptic input current (and therefore in σ), in addition to changes in discharge rate and ISI variability. Since alterations in the amount of input current induced concurrent changes in discharge rate, CoV_ISI, and σ, it is reasonable to expect that synchronization indices did not vary monotonically with discharge rate and ISI variability, as indeed observed experimentally.

Another key observation of the present study is that the behaviour of ρ, CIS, and E with discharge rate and CoV_ISI varied greatly among MU pairs. This high variability in the relation between correlation measures and MU properties (discharge rate and CoV_ISI) is predicted by the theoretical formulations and is explained by the fact that correlation measures depends on the statistics of the input current, which are different for each motor neuron and thus for each MU pair. Additionally, motor neurons also differ in their intrinsic properties, which make the scaling factor S(DR) relating the output and input correlation [defined in Eq. (2)] to vary among pairs. Since the statistics of the input current and motor neuron intrinsic properties cannot be estimated in vivo, the factor S(DR) associated to each MU pair is unknown and, as a result, the variability in output correlation measures among MU pairs is large.

4.3 Correlation of MU spike trains and common input

In the present study, we have demonstrated a relation between synchronization indices and discharge rate and its variability, indicating that the measures of output correlation are not dictated solely by the strength of common input. The non-monotonic increase of ρ, CIS, and E with discharge rate (observed for individual MU pairs) has given further support to the dependence of the output correlation on the statistics of the input current. Moreover, this dependence cannot be eliminated easily, as it is due to a limitation imposed by the low sampling frequency of alpha motor neurons (Negro and Farina, 2012). Therefore, the concurrent change of both input correlation and statistics of synaptic current would result in variations of the estimated output correlation, limiting the possibility of inferring definite conclusions about the strength of common synaptic input in pairs of motor neurons. This means that the output correlation between pairs of MU spike trains is not linearly related to the common input that the motor neurons receive. An important implication of this result is that an attempt to determine the relation between the input correlation and variables that change the discharge rate, such as force (Schmied and Descarreaux 2010), is challenging because it is not possible to distinguish between the variations of input correlation and statistics of the total synaptic currents. A second implication is that the measures of output correlation from pairs of MUs cannot be used to infer conclusions on the degree of common input that

the respective motor neurons receive. Nonetheless, the presence of a significant output correlation indicates the necessary presence of common input.

The present results have emphasized the complexity of the relation between the output (measured) correlation and input (actual) correlation to motor neurons. This complexity underlines that inferring the strength of common input (or the amount of anatomical connections shared by motor neurons) from output correlation measures is not possible. This conclusion is different from stating that synchronous MU firing instances are not caused by common inputs, as argued by De Luca and Kline (2016). We argue that the observation of a statistically significant correlation between MU firings indicates the presence of common input and that, conversely, the absence of significant correlation between spike trains of a proportion of MU pairs does not necessarily implies the absence of common input (Boonstra et al., 2016). The latter claim is based on the fact that common input may not necessarily determine correlated firings in some MU pairs due to the non-linearity of the relation between output and input correlation.

Finally, it should be emphasized that the correlations found between ρ and the two normalized indices studied (CIS and E) were high (>0.7), indicating that a direct measure of the magnitude of the peak of the cross-correlation histogram was closely related to other (more sophisticated) normalized measures of such peak. In addition, our data showed that the dependence of ρ on discharge rate and ISI variability was essentially similar to the dependence of CIS and E on these MU properties. Taken together, these findings suggest that ρ provides similar information as normalized indices.

In conclusion, we have demonstrated that measures of output correlation estimated from motor unit spike trains (in particular, the raw output correlation, the common input strength, and the synchronous impulse probability) were positively related to discharge rate and negatively related to inter-spike interval variability, with a large variability in these relations. Moreover, analysis of individual motor unit pairs showed that output correlation measures were not linearly related with discharge rate, and that the behaviour of these output correlation measures with discharge rate varied greatly among MU pairs. Collectively, these results indicate that, despite the synchronization between motor unit firing trains is necessarily caused by the common input to motor neurons, the output correlation between pairs of motor units cannot be used to quantify common input.

GRANTS

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FINANCIAL DISCLOSURE

The authors declare that they have no financial interests.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

References

Boonstra TW, Farmer SF, Breakspear M. Using Computational Neuroscience to Define Common Input to Spinal Motor Neurons. *Front Hum Neurosci* 10: 313, 2016.

Bremner FD, Baker JR, Stephens JA. Variation in the degree of synchronization exhibited by motor units lying in different finger muscles in man. *J Physiol* 432: 381–99, 1991.

Christou EA, Rudroff T, Enoka JA, Meyer F, Enoka RM. Discharge rate during lowforce isometric contractions influences motor unit coherence below 15 Hz but not motor unit synchronization. *Exp Brain Res* 178: 285–95, 2007.

Datta AK, Stephens JA. Synchronization of motor unit activity during voluntary contraction in man. *J Physiol* 422: 397–419, 1990.

de la Rocha J, Doiron B, Shea-Brown E, Josic K, Reyes A. Correlation between neural spike trains increases with firing rate. *Nature* 448: 802–806, 2007.

De Luca CJ, Roy AM, Erim Z. Synchronization of motor-unit firings in several human muscles. *J Neurophysiol* 70: 2010–23, 1993.

De Luca CJ, Kline JC. Statistically rigorous calculations do not support common input and long-term synchronization of motor-unit firings. *J Neurophysiol* 112(11):2729-44, 2014.

De Luca CJ, Kline JC. The common input notion, conceived and sustained by conjecture. *J Neurophysiol* 115(2):1079-80, 2016.

Ellaway PH. Cumulative sum technique and its application to the analysis of peristimulus time histograms. *Electroencephalogr Clin Neurophysiol* 45: 302–4, 1978.

Ellaway PH, Murthy KS. The origins and characteristics of cross-correlated activity between gamma-motor neurones in the cat. *Q J Exp Physiol* 70: 219–32, 1985.

Farmer SF, Swash M, Ingram DA, Stephens JA. Changes in motor unit synchronization following central nervous lesions in man. *J Physiol* 463: 83–105, 1993.

Fling BW, Christie A, Kamen G. Motor unit synchronization in FDI and biceps brachii muscles of strength-trained males. *J Electromyogr Kinesiol* 19: 800–9, 2009.

Heckman C, Enoka RM. Physiology of the motor neuron and the motor unit. *Handbook of Clinical Neurophysiology* 4: 119–147, 2004.

Ishizuka N, Mannen H, Hongo T, Sasaki S. Trajectory of group Ia afferent fibers stained with horseradish peroxidase in the lumbosacral spinal cord of the cat: three dimensional reconstructions from serial sections. *J Comp Neurol* 186: 189–211, 1979.

Kamen G, Roy A. Motor unit synchronization in young and elderly adults. *Eur J Appl Physiol* 81(5): 403-410, 2000.

Kirkwood PA, Sears TA. The synaptic connexions to intercostal motor neurones as revealed by the average common excitation potential. *J Physiol* 275: 103–134, 1978.

Kirkwood PA, Sears TA, Tuck DL, Westgaard RH. Variations in the time course of the synchronization of intercostal motoneurones in the cat. *J Physiol* 327:105-35, 1982.

Kirkwood PA. On the use and interpretation of cross-correlations measurements in the mammalian central nervous system. *J Neurosci Methods* 1: 107–132, 1979.

Kline JC, De Luca CJ. Synchronization of motor unit firings: an epiphenomenon of firing rate characteristics not common inputs. *J Neurophysiol* 1:115(1):178-92, 2016.

Lawrence DG, Porter R, Redman SJ. Corticomotor neuronal synapses in the monkey: light microscopic localization upon motor neurons of intrinsic muscles of the hand. *J Comp Neurol* 232: 499–510, 1985.

Lemon RN. Descending pathways in motor control. *Annu Rev Neurosci* 31: 195–218, 2008.

Logigian EL, Wierzbicka MM, Bruyninckx F, Wiegner AW, Shahahi BT, Young RR. Motor unit synchronization in physiologic, enhanced physiologic, and voluntary tremor in man. Ann Neurol 23(3): 242-50, 1988.

McGill KC, Lateva ZC, Marateb HR. EMGLAB: an interactive EMG decomposition program. *J Neurosci Methods* 149: 121–133, 2005.

Mori S. Entrainment of motor-unit discharges as a neuronal mechanism of synchronization. *J Neurophysiol* 38(4): 859-870, 1975.

Negro F, Farina D. Decorrelation of cortical inputs and motor neurone output. *J Neurophysiol* 106: 2688–2697, 2011.

Negro F, Farina D. Factors influencing the estimates of correlation between motor unit activities in humans. *PLoS One* 7(9):e44894, 2012.

Nordstrom MA, Fuglevand AJ, Enoka RM Estimating the strength of common input to human motor neurons from the cross-correlogram. *J Physiol* 453: 547–574, 1992.

Rodriguez-Falces J, Negro F, Gonzalez-Izal M, Farina D. Spatial distribution of surface action potentials generated by individual motor units in the human biceps brachii muscle. *J Electromyogr Kinesiol* 23(4):766-77, 2013.

Schmied A, Ivarsson C, Fetz EE. Short-term synchronization of motor units in human extensor digitorum communis muscle: relation to contractile properties and voluntary control. *Exp Brain Res* 97(1):159-72, 1993.

Schmied A, Vedel JP, Pagni S. Human spinal lateralization assessed from motor neurone synchronization: dependence on handedness and motor unit type. *J Physiol* 480: 369–387, 1994.

Schmied A, Descarreaux M. Influence of contraction strength on single motor unit synchronous activity. *Clin Neurophysiol*. 121(10):1624-32, 2010.

Sears TA, Stagg D. Short-term synchronization of intercostal motor neurone activity. *J Physiol* 263: 357–381, 1976.

Semmler JG, Steege JW, Kornatz KW, Enoka RM. Motor-unit synchronization is not responsible for larger motor-unit forces in old adults. *J Neurophysiol* 84(1): 358-66, 2000.

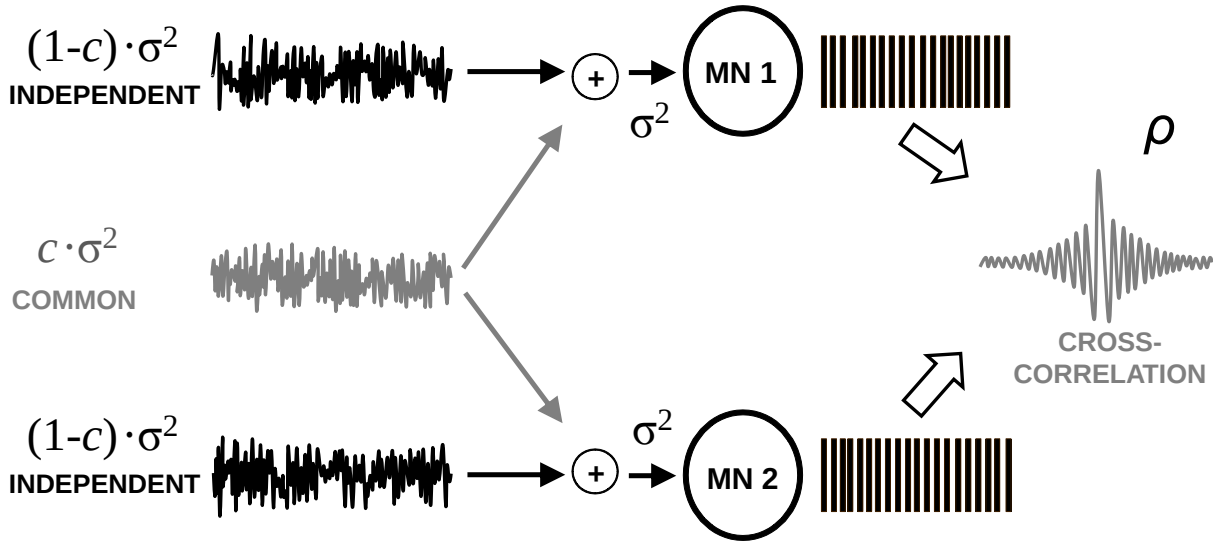
Semmler JG, Sale MV, Meyer FG, Nordstrom MA. Motor-unit coherence and its relation with synchrony are influenced by training. *J Neurophysiol* 92: 3320–3331, 2004.

Semmler JG, Nordstrom MA, Wallace CJ. Relationship between motor unit short-term synchronization and common drive in human first dorsal interosseous muscle. *Brain Res* 767: 314–320, 1997.

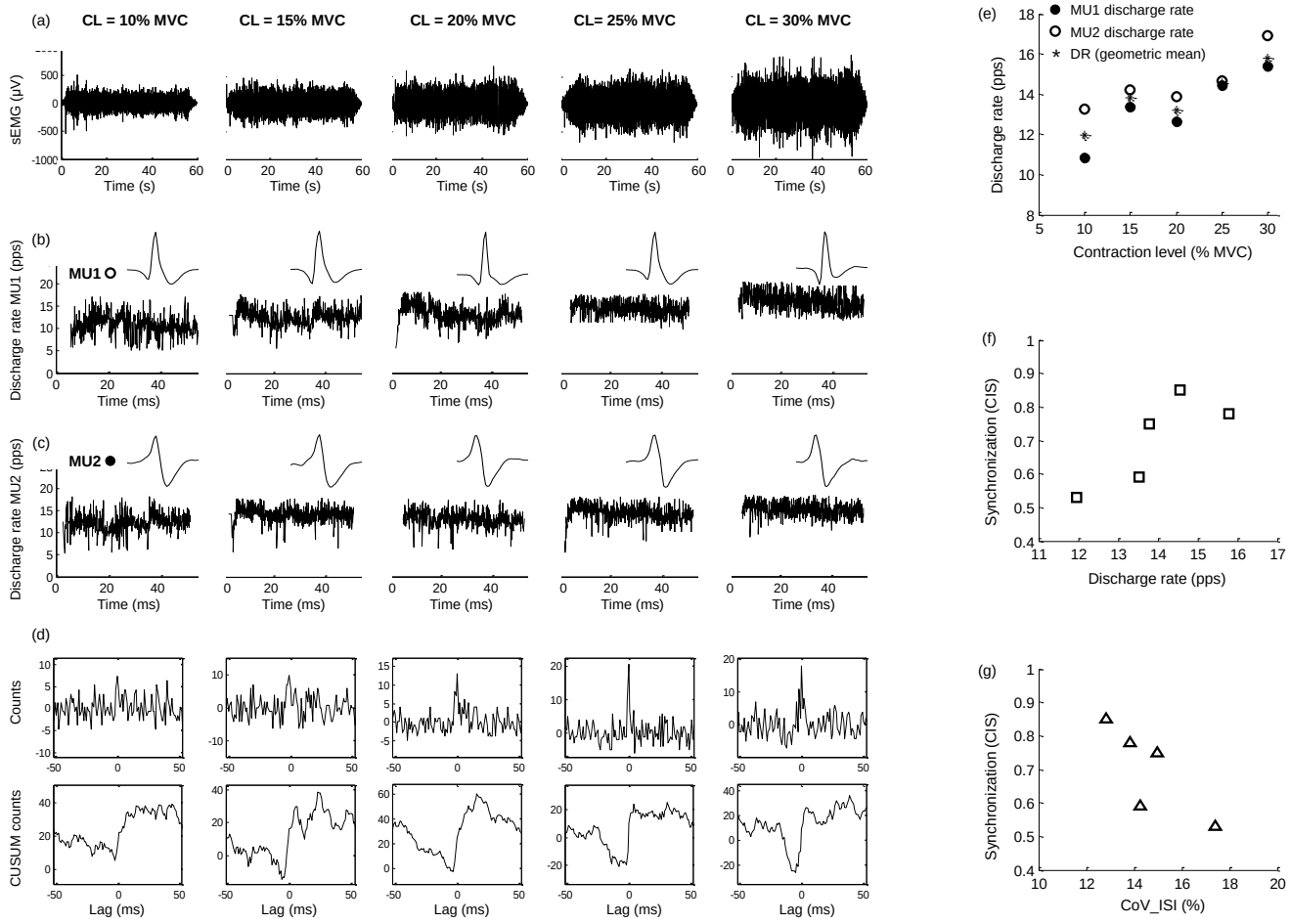
Türker KS, Powers RK. Effects of common excitatory and inhibitory inputs on motor neuron synchronization. *J Neurophysiol* 86: 2807– 2822, 2001.

Türker KS, Powers RK. The effects of common input characteristics and discharge rate on synchronization in rat hypoglossal motoneurones. *J Physiol* 15: 245-60, 2002.

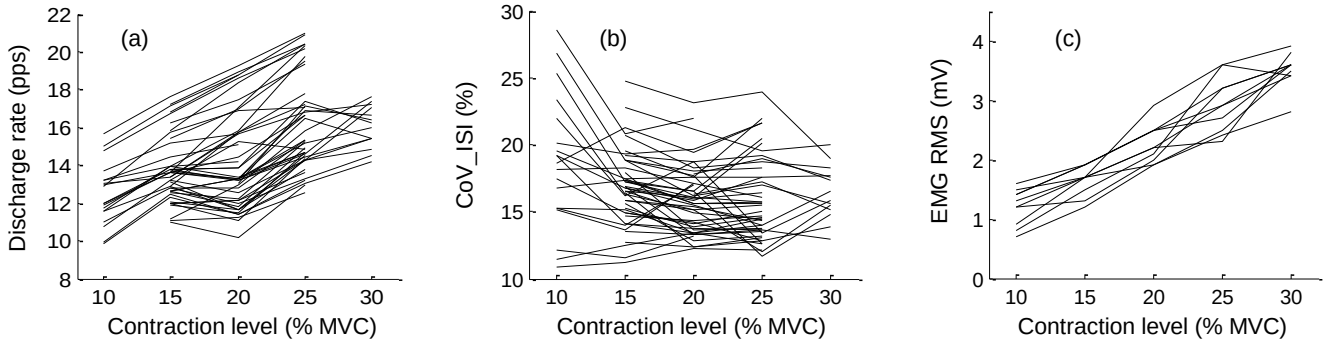
FIGURES



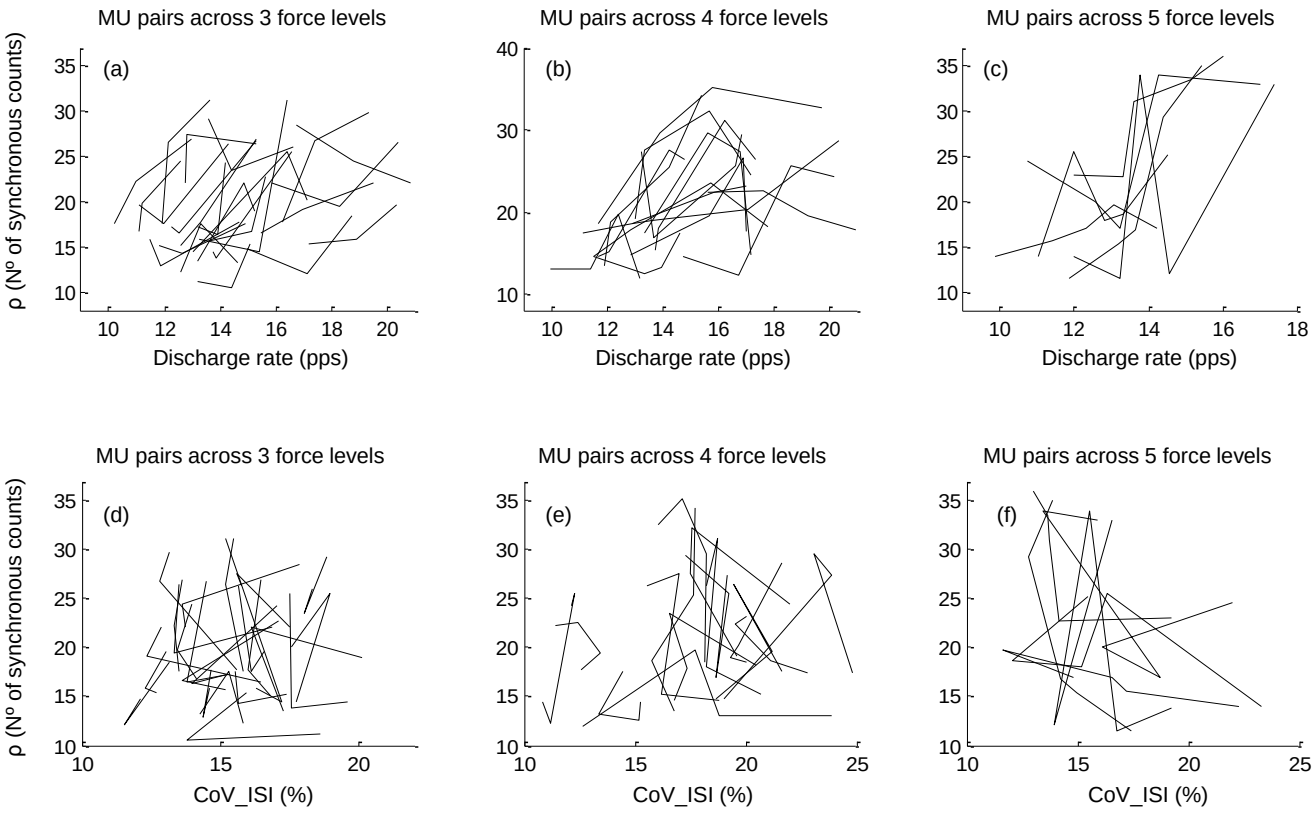
(Fig. 1)


(a)
CL = 10% MVC
CL = 15% MVC
CL = 20% MVC
CL= 25% MVC
CL = 30% MVC
sEMG (μV)
Time (s)
(b)
MU1
Discharge rate MU1 (pps)
Time (ms)
(c)
MU2
Discharge rate MU2 (pps)
Time (ms)
(d)
Counts
CUSUM counts
Lag (ms)
(e)
MU1 discharge rate
MU2 discharge rate
DR (geometric mean)
Discharge rate (pps)
Contraction level (% MVC)
(f)
Synchronization (CIS)
Discharge rate (pps)
(g)
Synchronization (CIS)
CoV_ISI (%)

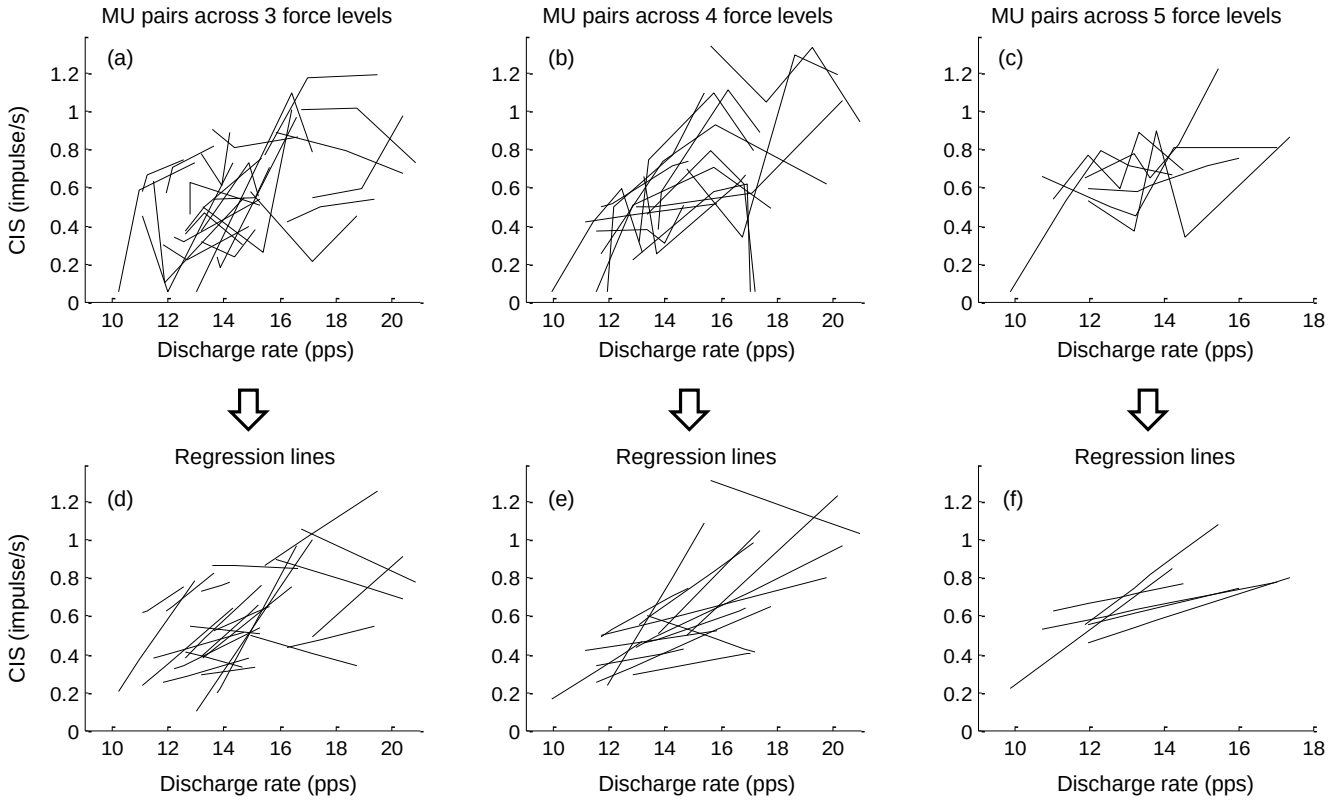
(Fig. 2)


(a)
Discharge rate (pps)
Contraction level (% MVC)
(b)
CoV_ISI (%)
Contraction level (% MVC)
(c)
EMG RMS (mV)
Contraction level (% MVC)

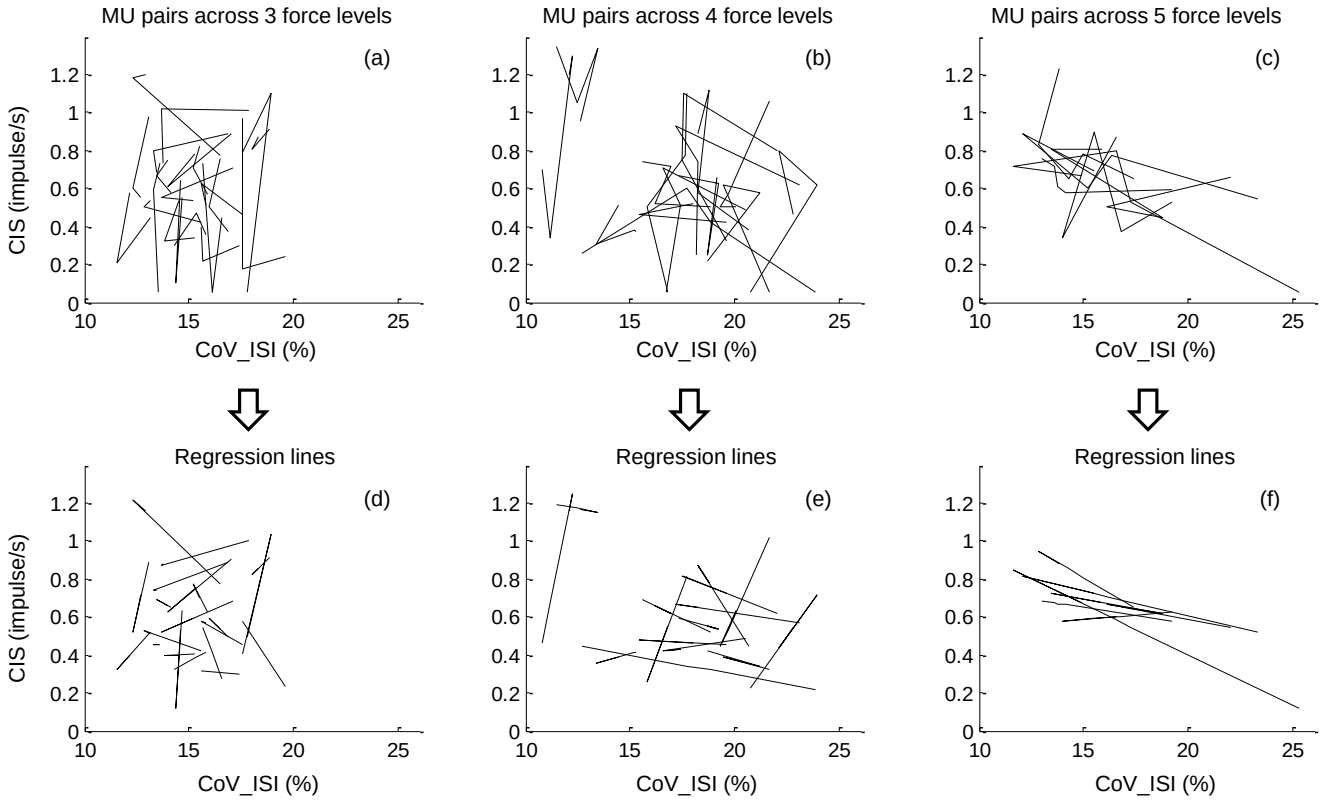
(Fig. 3)


MU pairs across 3 force levels
MU pairs across 4 force levels
MU pairs across 5 force levels
(a)
(b)
(c)
(d)
(e)
(f)
ρ (Nº of synchronous counts)
Discharge rate (pps)
CoV_ISI (%)

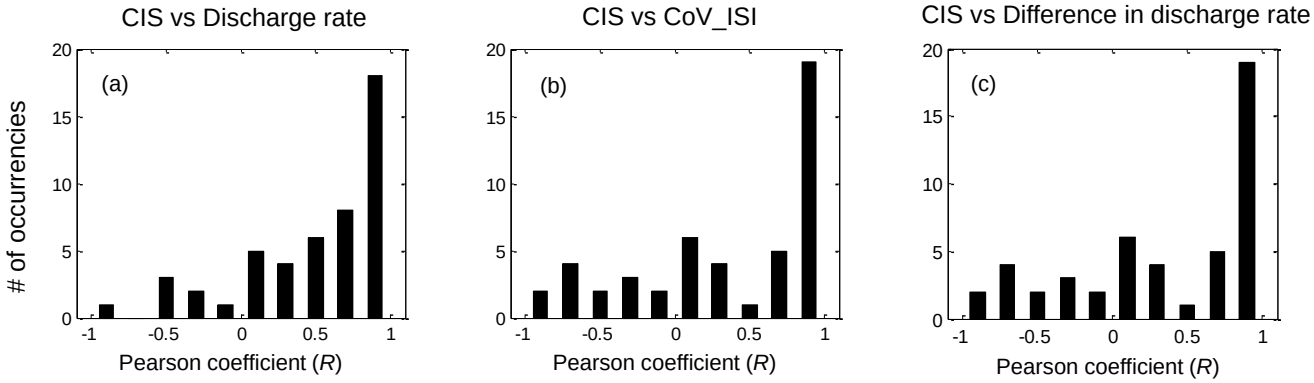
(Fig. 4)


MU pairs across 3 force levels
MU pairs across 4 force levels
MU pairs across 5 force levels
(a)
(b)
(c)
CIS (impulse/s)
Discharge rate (pps)
Regression lines
(d)
(e)
(f)

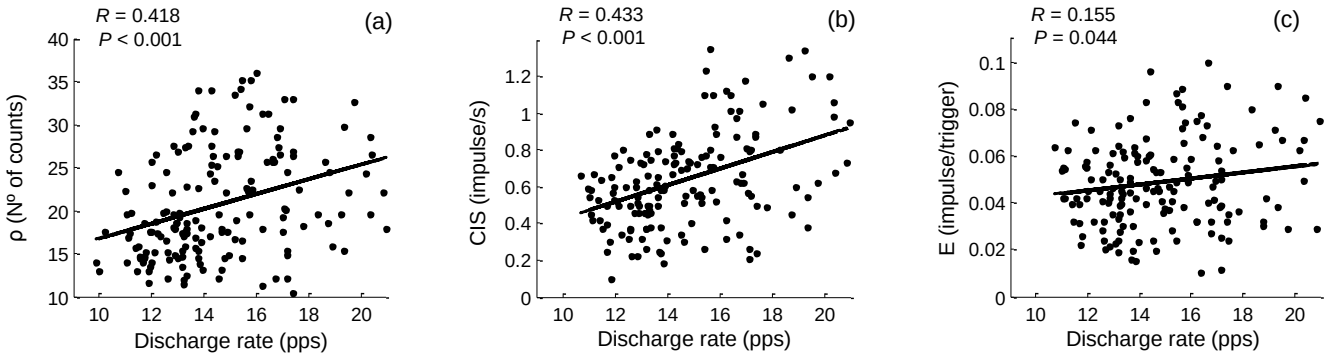
(Fig. 5)


MU pairs across 3 force levels
MU pairs across 4 force levels
MU pairs across 5 force levels
(a)
(b)
(c)
(d)
(e)
(f)
CIS (impulse/s)
CoV_ISI (%)
Regression lines
0
0.2
0.4
0.6
0.8
1
1.2
10
15
20
25

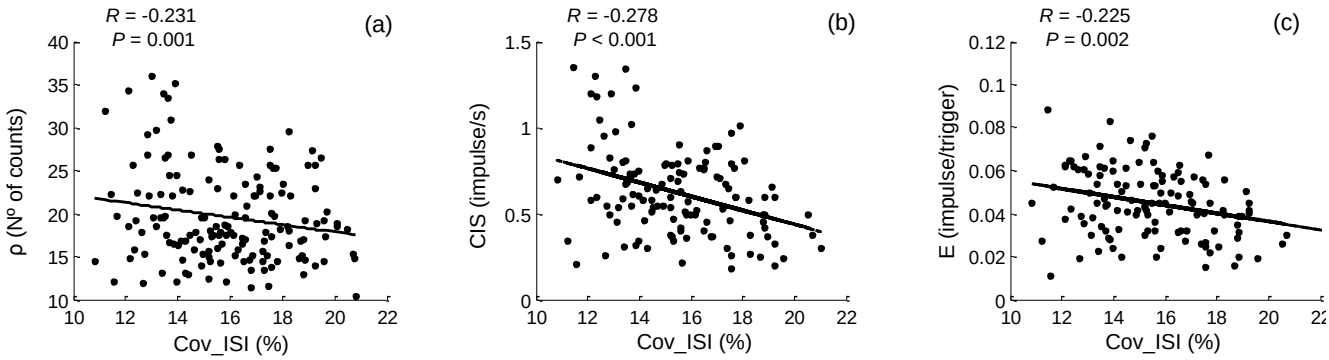
(Fig. 6)


CIS vs Discharge rate
CIS vs CoV_ISI
CIS vs Difference in discharge rate
# of occurrencies
(a)
(b)
(c)
0
5
10
15
20
-1
-0.5
0
0.5
1
Pearson coefficient (R)

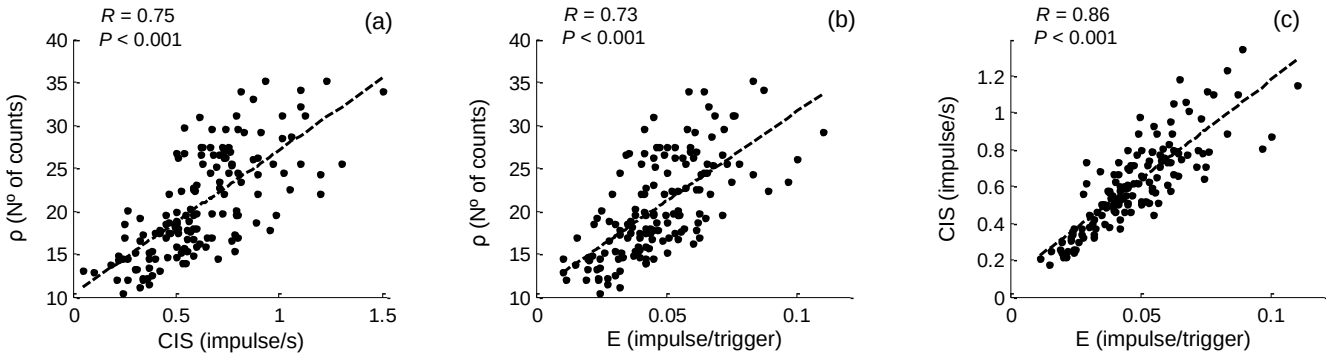
(Fig. 7)


R = 0.418
P < 0.001
(a)
ρ (Nº of counts)
Discharge rate (pps)
R = 0.433
P < 0.001
(b)
CIS (impulse/s)
Discharge rate (pps)
R = 0.155
P = 0.044
(c)
E (impulse/trigger)
Discharge rate (pps)

(Fig. 8)


R = -0.231
P = 0.001
(a)
ρ (Nº of counts)
Cov_ISI (%)
R = -0.278
P < 0.001
(b)
CIS (impulse/s)
Cov_ISI (%)
R = -0.225
P = 0.002
(c)
E (impulse/trigger)
Cov_ISI (%)

(Fig. 9)


R = 0.75
P < 0.001
(a)
ρ (Nº of counts)
CIS (impulse/s)
R = 0.73
P < 0.001
(b)
ρ (Nº of counts)
E (impulse/trigger)
R = 0.86
P < 0.001
(c)
CIS (impulse/s)
E (impulse/trigger)

(Fig. 10)

Parameters	Min vs. Max discharge rate			Min vs. Max force			Min vs. Max CoV_ISI		
	Minimum	Maximum	*P*-value	Minimum	Maximum	*P*-value	Minimum	Maximum	*P*-value
EMG RMS (mV)	1.1 (0.3)	3.2 (0.5)	<0.001	1.1 (0.3)	3.3 (0.4)	<0.001	2.8 (0.5)	1.6 (0.4)	<0.001
Flexion force (N)	21.2 (3.1)	57.4 (4.5)	<0.001	20.5 (3.4)	58.9 (4.9)	<0.001	53.4 (4.4)	23.7 (3.6)	<0.001
Discharge rate (pps)	12.85 (1.78)	16.52 (2.29)	<0.001	12.99 (1.71)	16.47 (2.30)	<0.001	15.29 (1.85)	14.27 (2.97)	0.018
CoV_ISI (%)	18.1 (3.6)	16.3 (3.1)	0.007	17.8 (3.4)	16.4 (3.2)	0.023	15.1 (2.5)	18.5 (3.4)	<0.001
Peak width (ms)	9.56 (3.2)	8.73 (3.2)	0.212	9.25 (3.0)	9.06 (3.3)	0.775	8.01 (3.3)	9.35 (3.2)	0.019
ρ	14.3 (2.1)	25.7 (2.9)	<0.001	14.9 (2.3)	26.8 (2.7)	<0.001	19.8 (2.3)	18.8 (2.5)	0.002
CIS (impulse/s)	0.46 (0.27)	0.70 (0.27)	<0.001	0.50 (0.34)	0.67 (0.33)	<0.001	0.66 (0.26)	0.58 (0.32)	0.097
E (impulse/trigger)	0.038 (0.021)	0.050 (0.019)	<0.001	0.041 (0.024)	0.048 (0.022)	0.002	0.049 (0.018)	0.044 (0.021)	0.118

(Table 1)

MULTIPLE REGRESSION ANALYSIS	DEPENDENT VARIABLES	ρ	CIS	SIP
Determination coefficient (R^2)		0.158	0.196	0.103
INDEPENDENT VARIABLES				
Discharge rate	P-value	<0.001	<0.001	0.022
CoV_ISI	P-value	0.048	0.023	0.043
Difference in discharge rate between motor neurons	P-value	0.823	0.792	0.653

(Table 2)

CAPTIONS

Fig. 1 Schematic diagram of the relation between the output correlation, calculated from the output spike trains of two MUs (ρ), and the independent and common input (c) currents driven to two motor neurons (MNs). The symbol σ represents the standard deviation of the input current.

Fig. 2 (a) Time plots of surface electromyographic (sEMG) recordings resulting from 60-s isometric contractions of the biceps brachii at five contraction forces. Two motor units (MU1 ● and MU2 ○) were detected and tracked across the five contraction levels. The time plots of the discharge rates of MU1 and MU2 during the above contraction intervals are shown in (b) and (c), respectively. The intramuscular potential associated to each firing train is shown above each plot. Discharge rate increased with contraction strength (e). For each contraction level, the cross-correlation histogram (and the associated CUSUM diagram) between the firing trains of MU1 and MU2 were built (d) to determine the degree of synchronization (evaluated in this example by the common input strength, CIS) between these MUs. The synchronization index analysed (common input strength, CIS) tended to increase with discharge rate (f) and to decrease with the coefficient of variation of the interspike-intervals (CoV_ISI) (f).

Fig. 3 Lines representing the changes in discharge rate (a) and coefficient of variation of the interspike-interval (CoV_ISI) (b) as a function of force level for the 48 pairs of MUs. Lines in (c) represent the changes in EMG RMS with force level for the 10 subjects.

Fig. 4 Lines representing the changes in output correlation (ρ) as a function of discharge rate (upper panels) and the coefficient of variation of the interspike-interval (CoV_ISI) (lower panels) for 48 pairs of MUs. Each line connects, for a given MU pair, the ρ values obtained at different discharge rates. Plots in the first, second, and third columns contain the lines of MU pairs tracked across 3, 4, and 5 force levels, respectively.

Fig. 5 Upper panels - Lines representing the changes in common input strength (CIS) with discharge rate for 48 pairs of MUs. Each line connects, for a given MU pair, the CIS values obtained at different discharge rates. Plots in the first, second, and third columns contain the lines of MU pairs tracked across 3, 4, and 5 force levels, respectively. Lower panels – Linear regression lines of best fit calculated from the above CIS-discharge rate data.

Fig. 6 Upper panels - Lines representing the changes in common input strength (CIS) with the coefficient of variation of the interspike-interval (CoV_ISI) for 48 pairs of MUs. Each line connects, for a given MU pair, the CIS values obtained at different CoV_ISI. Plots in the first, second, and third columns contain the lines of MU pairs tracked across 3, 4, and 5 force levels, respectively. Lower panels – Linear regression lines of best fit calculated from the above CIS-CoV_ISI data.

Fig. 7 Histograms of the Pearson correlation coefficient (*R*) obtained from the relationships between the CIS index and discharge rate (a), between the CIS index and the interspike-interval variability (CoV_ISI) (b), and between the CIS index and the difference in discharge rate between pairs of motor neurons (c) calculated from 48 MU pairs.

Fig. 8 Pooled values of output correlation, ρ (a), common input strength, CIS (b), and synchronous impulse probability, E (c), obtained from the 48 MU pairs plotted as a function of discharge rate. The straight line represents the linear regression line of best fit associated with the coefficient of correlation (*R*) level significance (*P*).

Fig. 9 Pooled values of output correlation, ρ (a), common input strength, CIS (b), and synchronous impulse probability, E (c), obtained from the 48 MU pairs plotted as a function of the coefficient of variation of the interspike-interval (CoV_ISI). The straight line represents the linear regression line of best fit associated with the coefficient of correlation (*R*) level significance (*P*).

Fig. 10 Scatter plots of output correlation vs common input strength (ρ vs CIS, a), output correlation vs synchronous impulse probability, (ρ vs E, b), and common input strength vs synchronous impulse probability, (CIS vs E, b) obtained from the 48 MU pairs. In each plot, the straight line represents the linear regression line of best fit associated with the coefficient of correlation (*R*) level significance (*P*).

Table 1. Mean (SD) values of EMG amplitude, force, discharge rate, peak width of the cross-correlogram, common input strength (CIS), and synchronous impulse probability (E) measured at minimum and maximum discharge rates (left) and at minimum and maximum force levels (centre), and minimum and maximum coefficient of variation of the interspike-interval (CoV_ISI) (right).

Table 2. Results of multiple regression analysis to explain variation in synchronization indices: dependent variables = ρ, CIS, and E; independent variables = discharge rate, CoV_ISI, difference in discharge rate of motor neurons.